Dr Butler.

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OBSERVATIONS ON THE BACTERIAL AND FUNGAL FLORA OF THE UPPER AIR

BY

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REPRINTED FROM THE

TRANSACTIONS OF THE BRITISH MYCOLOGICAL SOCIETY

VOL. XIV, PARTS I AND II, 11 MARCH 1929

CAMBRIDGE

AT THE UNIVERSITY PRESS

PRINTED IN GREAT BRITAIN

[Reprinted from the
TRANSACTIONS OF THE BRITISH MYCOLOGICAL SOCIETY
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OBSERVATIONS ON THE BACTERIAL AND FUNGAL FLORA OF THE UPPER AIR.

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This paper records some work that was carried out in spore-trapping experiments from airplanes. The flights were made from Duxford in machines belonging to the Cambridge University Air Squadron. The work was started at the end of 1926 and since that date various types of spore-trap have been used. The simplest method employed was to expose in the air microscope slides (3 inches by 1 inch) smeared with vaseline; these were exposed by hand from the cockpit. Another method, but more difficult in operating, was by means of photographic slide cases fixed in a brass clamp on the upper wing above the cockpit. Each case contained two quarter plates smeared with vaseline

and the exposure was made by withdrawing the slide of the case for a given time; the reverse side of the case contained a similar plate which acted as a control. A somewhat similar method consisted in exposing the same type of photographic slide cases by hand from the cockpit. Another method which was tried was by exposing slides in a mechanical spore-trap attached to the end of the wing; this was very similar to that described by Stakman, Henry, Curran and Christopher*. It was operated in the same way from the cockpit.

When the slide cases were taken to the laboratory they were opened quickly and the plates immediately covered with large cover slips. These different types of spore-traps were operated by Mr Everett of the Hampshire Light Aero Club and Lieut. Goddard of the Cambridge University Air Squadron. The objections to the above methods, in my opinion, are these: the chance that the slide may become contaminated when applying the vaseline, the difficulty of insuring that the interiors of the slide cases are not contaminated before and after exposure, the risk of contaminating the slides when placing on the cover slips and lastly the difficulty of identifying the spore so obtained. Where a few spores only are obtained on a plate there is always the just criticism to be met that these slides were contaminated prior to or after exposure. On this account I abandoned these methods and decided not to communicate the results obtained. The trend of these experiments showed, however, that the upper air contained a large quantity of spores. *En passant* I consider one of the best methods yet adopted to be that of Stakman, Henry, Gordon, and Christopher who place inside a bottle a microscope slide attached to a wooden paddle, the upper part of which extends through a hole in the cork of the bottle and serves as a handle, after exposure the slide is again placed in the bottle. Unfortunately, as these authors state, the method is very cumbersome.

The method I finally adopted was to expose culture media in (*a*) test tubes, (*b*) Petri dishes. The exposures were made at the altitude required and then sealed and at a later date examined for contaminations. For the very general observations that were made this method was serviceable. In one flight an exhausted flask containing potato agar was taken up with the intention of exposing it above 10,000 feet; since, however, this altitude was not reached the flask was not exposed and was taken back to the laboratory. During the night the flask burst (or collapsed) with some violence. It is possible that such a method would involve some risk since the glass would be subject to changing stresses.

* Spores in the Upper Air. Journ. Agric. Res. XXIV.

Table I shows the results of a series of flights which were carried out from Duxford Aerodrome and in which test tubes containing potato agar were exposed at various altitudes.

This table shows that approximately forty per cent. of the exposures were positive and illustrates the ubiquitous nature of microscopic plant life, and shows what an excellent medium the air must be for the dissemination of pathogenic and saprophytic organisms.

Table II shows the results that were obtained on exposing Petri dishes containing potato agar.

The figures in this table show that spores and bacteria may be found at very great heights and yet still retain their vitality. It is interesting to note that in the flight on January 31st, 1928, the last dishes exposed at 9000 and 10,000 feet showed only bacterial colonies and that no fungi developed. It is very probable that bacteria and the lighter spores may be carried to much greater heights than this, and it seems reasonable to anticipate that in this rarer atmosphere the last living organisms would be bacteria.

A just criticism of these experiments would be that the plates when exposed, picked up spores attached to the airplane, which were released by the slip stream. This is a distinct possibility but any serious contamination appears unlikely for these reasons. The plates were held at arm's length from the cockpit and the plate faced between the struts so that it was approximately between the upper and lower wings. Again, the negative nature of the exposures at 13,000 feet is evidence itself against any serious contamination of this nature. In this last connection it is interesting to note that Stakman, Henry, Curran, and Christopher have obtained spores at a height as great as 16,500 feet. They state that "The highest altitude at which slides were exposed was 16,500 feet or more than 3 miles above the surface of the earth. This slide was exposed between Waco and San Antonio, Tex. on April 28th, 1921, and two uredinio-spores of what appeared to be *Puccinia triticina* were caught. It would not be safe, however, to conclude that spores normally occur at this height, as only a few flights were made at such extreme elevations. Spores are very numerous up to about 11,000 feet but at higher altitude they apparently are relatively scarce."

In Table III are given the results obtained when Petri dishes containing potato agar were exposed below, in, and above the clouds.

This table shows that in these flights there was a higher bacterial and fungal flora in the clouds than above or below them. It is probable that spores may form surfaces for condensation of water vapour. It would be anticipated that there

would be a higher bacterial and fungal flora in clouds than immediately above or below them. In considering Table III the relative heights at which the exposures were made should also be taken into account. Generally speaking the nearer the earth the higher will be the bacterial and fungal flora. For example, in the flight made on May 29th, 1928, a plate exposed for one minute at 1500 feet (not quoted in the table) showed after seven days the presence of thirty-five fungi and sixty-two bacterial colonies.

Table I.

Date	Altitude (in ft.) at which exposures were made	Time of exposure in seconds	Negative (N) or positive (P) after 7 days	Negative (N) or positive (P) after 21 days
14. x. 27	1,000	30	—	N
	1,000	30	—	N
	1,000	30	—	N
17. x. 27	1,000	30	—	N
	1,500	30	—	N
	2,000	30	—	P
	3,000	30	—	N
	4,000	30	—	N
	5,000	30	—	N
	5,500	30	—	P
	6,000	30	—	P
	6,000	30	—	N
	6,000	30	—	N
26. x. 27	1,000–1,500	30	—	N
	1,000–1,500	30	—	P
	1,000–1,500	30	—	P
	1,000	30	—	P
	1,500	30	—	N
	2,000	30	—	P
	2,500	30	—	N
	2,500	30	—	N
	2,500	30	—	N
4. xi. 27	500	30	—	P
	600	30	—	P
	600	30	—	P
	600	30	—	N
	700	30	—	P
	700	30	—	P
	800	30	—	P
	800	30	—	P
	900	30	—	N
	900	30	—	N
7. xi. 27	2,000	30	—	P
	2,000	30	—	N
	2,000	30	—	N
	2,000	30	—	P
	2,000	30	—	P
	2,000	30	—	P
	1,500	30	—	N
	1,500	30	—	P
	1,700	30	—	N
	1,800	30	—	N

Table I (*cont.*)

Date	Altitude (in ft.) at which exposures were made	Time of exposure in seconds	Negative (N) or positive (P) after 7 days	Negative (N) or positive (P) after 21 days
17. i. 28	3,500	30	P	P
	5,000	30	N	N
	6,500	30	N	P
	7,500	60	N	P
	8,500	60	N	N
	10,000	300	N	N
31. i. 28	1,000–1,200	60	P	—
	3,000	60	N	—
	5,000	60	N	—
	6,500–7,000	60	N	—
	8,000–8,500	60	N	—
	9,000	60	N	—
	9,500	60	N	—
	10,000	60	N	—
	10,000	60	N	—
	11,000	60	N	—
2. ii. 28	1,000–2,000	60	N	—
	2,000–3,000	60	P	—
	3,000–4,000	60	N	—
	4,500–5,000	60	N	—
	5,000–5,500	60	P	—

An interesting flight was made from Duxford to Cambridge at an altitude of 2000 feet on May 20th; the sky was cloudless. Three plates were exposed for a period of one minute. On arrival at Cambridge the machine was put up to 4000 feet and the return journey made, three exposures being taken. A seventh plate was exposed as the machine was descending from 4000 feet to the aerodrome. After five days these Petri dishes were examined but it was impossible to count the number of contaminations on the dishes exposed on the outward journey. The dishes were literally covered with bacterial and fungal growths and many hundreds of spores and bacteria must have alighted on the medium. The same was true of the Petri dish exposed in descending from 4000 feet to the aerodrome. The plates exposed on the return journey at 4000 feet were much less heavily contaminated but these contaminations were greater than those obtained in previous flights.

If this flight be compared with those made in the winter months it is clear that in summer—or at least at the end of May—the air is very much more heavily charged with bacterial and fungal spores. The results obtained from these exposures render it easy to visualise how local epidemics of plant and animal diseases may be brought about.

Table II.

Date	Altitude	No. of mycelial growths which developed after 7 days	No. of bacterial colonies which developed after 7 days*	Time of exposure in seconds
31. i. 28	2,000	10	3	60
	4,000	7	11	60
	6,000	3	2	60
	8,000	1	0	60
	9,500	0	9	60
	10,500	0	6	60
2. ii. 28	1,000	14 (15)†	13 (25)†	30
	2,000	3 (3)	5 (5)	30
	3,000	4 (8)	8 (8)	30
	4,500	6 (6)	3 (3)	30
	6,000	0 (1)	1 (1)	30
	7,000	2 (3)	4 (5)	30
9. ii. 28	500	11	12	30
	500	7	5	30
	1,000	7	15	30
	1,500	14	15	30
	2,000	11	3	30
	2,000	5	5	30
	2,000	1	2	30
	2,500	3	7	30
	2,500	6	8	30
	2,500	8	6	30
	2,500	11	6	30
	2,500	3	5	30
22. ii. 28	2,000	7	7	30
	4,000	3	14	30
	6,000	6	7	30
	8,000	0	0	30
	9,000	2	1	30
	10,000	0	1	30
	12,000	3	1	30
	13,000	0	0	30
	13,000	0	0	30
	13,000	0	0	30

† Same plates examined after 14 days.

Discussion of Results.

The results show the ubiquitous nature of fungal spores and bacteria in the upper air and that a large number of these are viable even at an altitude of two miles above the earth. From the flights made it would seem clear that in the summer (as one would expect) the air is very much more heavily charged with fungal spores and bacteria than in the winter months. The trend of the work suggests that the air is the ideal medium for the

* I have since received a report from Mr H. J. Barker who kindly investigated some of these bateria. In a short summary of his results he states:—

"Nearly all the organisms are coloured types. This is usual in samples from the air. Of the twenty types isolated, eleven were bacteria (=non-sporing rods), two were bacilli (=sporing rods), four were cocci (including one sarcina), and three were Actinomycetes."

transportation of microscopic organisms and suggests that epidemics of plant and animal diseases may be brought about in this manner. ("It seems clear that there is a relationship between clouds or eddies of spores and localised heavy field infections of 'rust'":—Report of the Dominion Botanist for Canada, 1926, pp. 111.)

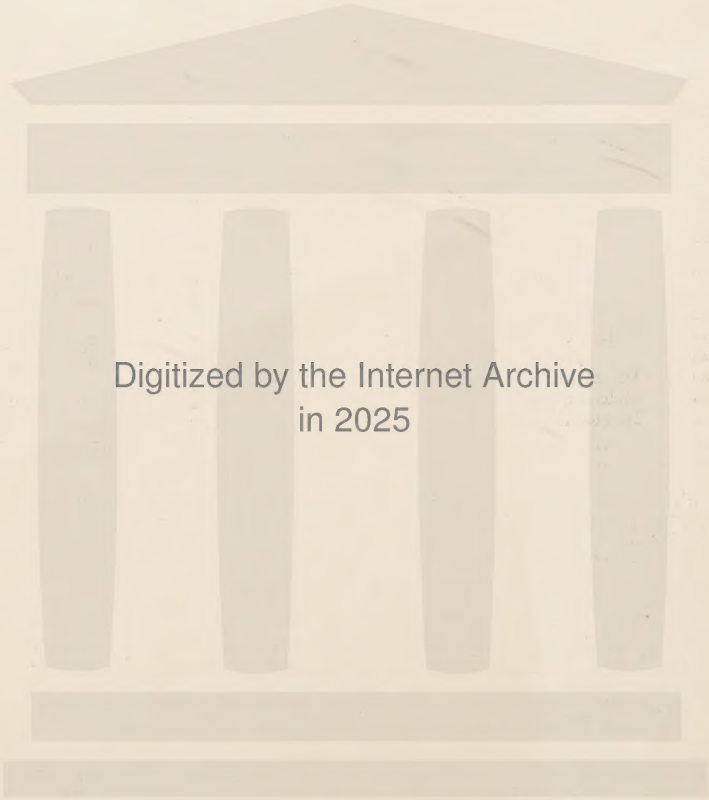
Table III.

Date	Altitude at which exposure was made	How exposed	Time of exposure in secs.	No. of fungi developing after 7 days	No. of bacterial colonies developing after 7 days	Remarks
7. iii. 28	2000	Exposed in slight drift of clouds	30	8	3	In clouds
	2000	,,	30	2	0	Bacterial
	4000	In clear air	30	0	0	Fungi colonies
	4400	In cloud bank	30	3	0	14 3
	4500	,,	30	1	0	Below or above
	4700	Above this cloud bank	30	0	0	clouds
	4800	,,	30	0	0	0 0
29. v. 28	3000	Below cloud	30	7	2	In clouds
	3000	,,	30	5	5	
	4500	In cloud	30	3	7	Bacterial
	4000	Below cloud	30	7	2	Fungi colonies
	3000	,,	30	16	5	60 64
	4000	In cloud	30	16	25	
	3500	Below cloud	30	10	1	Below cloud
	4000	In cloud	30	22	23	
	4000	,,	30	8	3	45 15
	4000	,,	30	11	9	

Some evidence has also been obtained that the clouds are more heavily charged with fungal spores and bacteria than the air a little below them. This is likely since spores would form suitable surfaces for the condensation of water vapour.

In one flight three plates were exposed at 13,000 feet and no development of fungi or bacteria took place. It is very probable, however, that viable organisms are to be met with considerably higher than this. The trend of the work suggests that at 10,000 feet the air is relatively free from microscopic organisms.

In conclusion the writer wishes to express his thanks to Group Captain Bowen, late Wing Commander of the Cambridge University Air Squadron, for his kindness and interest in the work and to Flight Lieutenant Goddard who has assisted him in ways too numerous to mention and from whom he has at all times received unqualified assistance. He also records his thanks to the Flight Instructors, Lieuts. French, Hordern and Nash, and finally to Mr Everett who co-operated in the first part of the work and carried out some of the experiments with various types of spore-traps.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